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## Claims

1. A method for the production of diphtheria toxin wherein a microorganism capable of producing diphtheria toxin is fermented using glucose as a carbon source,  
5 said method comprising adding glucose to a growing culture whereby the addition of glucose maintains a microorganism growth effective to support diphtheria toxin production.
- 10 2. A method as claimed in claim 1 wherein the diphtheria toxin is a mutated diphtheria toxin.
3. A method as claimed in claim 2 wherein the mutated  
15 diphtheria toxin has a cytotoxic A subunit.
4. A method as claimed in claim 3 wherein the mutated diphtheria toxin is CRM 107.
- 20 5. A method as claimed in any one of the preceding claims wherein the microorganism is corynebacterium diphtheriae.
6. A method as claimed in any one of the preceding  
25 claims wherein glucose is added in a fed batch method..
7. A method as claimed in claim 6 wherein the pH is maintained at between about 7.0 to about 7.5.
- 30 8. A method as claimed in any one of the preceding claims wherein the fermentation medium comprises from about 0.5 to about 1.5% yeast extract.
9. A method as claimed in any one of the preceding  
35 claims wherein the fermentation medium comprises no more than about 1% yeast extract.

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10. A method as claimed in any one of the preceding claims wherein the fermentation medium comprises cystine.

5 11. A method of purifying diphtheria toxin from a culture of toxin producing bacteria, said method comprising contacting a toxin containing preparation with an ion exchange matrix, eluting a fraction containing the toxin, applying the eluate to a  
10 hydrophobic matrix, and eluting a fraction containing the toxin.

12. A method of purifying diphtheria toxin from a culture of toxin producing bacteria said method  
15 comprising chromatographic steps of ion exchange chromatography and hydrophobic interaction chromatography, characterised in that said method comprises carrying out an ion exchange chromatography before hydrophobic interaction chromatography.

20 13. A method of purifying diphtheria toxin comprising (1) fermenting a microorganism strain capable of producing diphtheria toxin using glucose as a carbon source, said method comprising adding glucose to a  
25 growing culture whereby the addition of glucose maintains microorganism growth effective to support diphtheria toxin production; and (2) purifying the diphtheria toxin from the culture by contacting a toxin containing preparation derived  
30 therefrom with an ion exchange matrix, eluting a fraction containing the toxin, applying the eluate to a hydrophobic matrix, and eluting a fraction containing the toxin.

35 14. A method as claimed in any one of claims 11 to 13 wherein the toxin is produced by the method of any one of claims 1 to 10.

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15. A method as claimed in any one of claims 11 to 14 wherein the toxin containing preparation is a culture supernatant or a fraction thereof.
- 5 16. A method as claimed in any one of claims 11 to 15 wherein the toxin containing material is concentrated prior to ion exchange.
- 10 17. A method as claimed in any one of claims 11 to 16 wherein the ionic strength is reduced prior to ion exchange.
- 15 18. A method as claimed in any one of claims 11 to 17 wherein the diphtheria toxin is a mutant.
19. A method as claimed in claim 18 wherein the mutant toxin has a cytotoxic A subunit.
- 20 20. A method as claimed in claim 19 wherein the mutated toxin is CRM 107.
- 25 21. A method as claimed in any one of claims 11 to 20 wherein the microorganism is corynebacterium diphtheriae.
22. A method as claimed in any one of claims 1 to 4, or 6 to 20 wherein the microorganism is *E. coli*.
- 30 23. Diphtheria toxin having a purity of at least 98%.
24. Diphtheria toxin when produced by the method of any one of claims 1 to 22.
- 35 25. A method for preparing a diphtheria toxin conjugate comprising conjugating a diphtheria toxin produced by the method of any one of claims 1 to 22 with a cell specific binding or targeting moiety.

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26. A method as claimed in claim 25 wherein the cell specific binding or targetting moiety is transferrin.

5 27. A method of treatment of CNS neoplasm comprising administering to a subject a diphtheria toxin conjugate as produced by the method of claim 25 or claim 26.

10 28. The use of a diphtheria toxin conjugate produced by the method of any one of claims 25 or claim 26 in the manufacture of a medicament for use in the treatment of CNS neoplasm.